

Rec'd PCT/PTO 09 JUN 2005

PCT/EP 03114553

10/538000



Europäisches
Patentamt

European
Patent Office

Office européen
des brevets

REC'D 09 FEB 2004

WIPO

PCT

Bescheinigung

Certificate

Attestation

Die angehefteten Unterla-
gen stimmen mit der
ursprünglich eingereichten
Fassung der auf dem näch-
sten Blatt bezeichneten
europäischen Patentanmel-
dung überein.

The attached documents
are exact copies of the
European patent application
described on the following
page, as originally filed.

Les documents fixés à
cette attestation sont
conformes à la version
initialement déposée de
la demande de brevet
européen spécifiée à la
page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

02102819.6

**PRIORITY
DOCUMENT**
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

DEN HAAG, DEN
THE HAGUE,
LA HAYE, LE

29/01/04



Europäisches
Patentamt

European
Patent Office

Office européen
des brevets

**Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation**

Anmeldung Nr.:
Application no.:
Demande n°: **02102819.6**

Anmeldetag:
Date of filing:
Date de dépôt: **19/12/02**

Anmelder:
Applicant(s):
Demandeur(s):
**DSM IP Assets B.V.
6411 TE Heerlen
NETHERLANDS**

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:

Process for the production of a food product with reduced level of acrylamide

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat:
State:
Pays:

Tag:
Date:
Date:

Aktenzeichen:
File no.
Numéro de dépôt:

Internationale Patentklassifikation:
International Patent classification:
Classification internationale des brevets:

A23L1/03, A23L1/30, C12N9/82, C12N9/80

Am Anmeldetag benannte Vertragsstaaten:
Contracting states designated at date of filing:
Etats contractants désignés lors du dépôt:

AT/BG/BE/CH/CY/CZ/DE/DK/EE/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/

Bemerkungen:
Remarks:
Remarques:

**The applicant's name at the time of filing of the application was as follows:
DSM N.V.**

The registration of the change has taken affect from 26 June 2003 (26.06.2003)

NOVEL FOOD PRODUCTION PROCESS

The present invention relates to a process for the production of a food product involving at least one heating step and food products obtained thereof.

5

Acrylamide has been produced commercially for a long time for a variety of technical applications and therefore, its toxicological background is well evaluated. Acrylamide is used for the production of polyacrylamide, and the latter compound is applied in the production of drinking water, soil stabilization, industrial wastewater treatment, the winning of oil and laboratory applications.

10

Acrylamide is considered as probably carcinogenic for animals and humans. In 1991 the Scientific Committee on Food has investigated monomeric acrylamide in contact food materials and in its evaluation it was concluded that acrylamide is a genotoxic carcinogen. Bergmark *et al.* (Chem. Res. Toxicol., 10, 78-84 (1997)) demonstrated that acrylamide is also a component in tobacco smoke and this was the first link between the formation of acrylamide and the heating of biological material. Recently, the occurrence of acrylamide in a number of food and oven prepared foods was published (Tareke *et al.* Chem. Res. Toxicol. 13, 517-522. (2000)) and this resulted in world-wide concern. Further research revealed that considerable amounts of acrylamide are detectable in a variety of baked, fried and oven prepared common foods and it was demonstrated that the occurrence of acrylamide in food was the result of the baking process.

15

20

The official limit in the UK for acrylamide contamination in food products is set at 10 ppb (10 micrograms per kilogram) and the values presented above abundantly exceed this value for a lot of products, especially cereals, bread products and potato crisps.

25

The relation between the administered dose of acrylamide and tumor incidence was observed in animal tests in which rats were fed acrylamide via drinking water and which fate was followed during two years (Friedman, H.L. *et al.*), Fundam. Appl. Pharmacol. 85:154-168. M. (1986) and Johnson *et al.* Toxicol. Appl. Pharmacol. 85:154-168 (1986)). Chronic toxicity and oncogenicity study on acrylamide in incorporated in the drinking water of Fischer 344 rats.

30

When these data were combined with the results collected in Tareke *et.al.* in

which acrylamide bound to hemoglobin (N-(2-carbamoylethyl)valine) was studied as a function of an acrylamide containing diet to rats, it was calculated that the daily uptake of acrylamide is 1.6 ug acrylamide/kg, corresponding to a cancer risk of $7 \cdot 10^{-3}$ for humans from life-long exposure.

5 A pathway for the formation of acrylamide from amino acids and reducing sugars as a result of the Maillard reaction has been proposed Mottram *et al.* Nature 419:448. (2002). According to this hypothesis, acrylamide may be formed during the Maillard reaction. During baking and roasting, the Maillard reaction is mainly responsible for the color, smell and taste. A reaction associated with the Maillard is the Strecker
10 degradation of amino acids and a pathway to acrylamide was proposed. The formation of acrylamide became detectable when the temperature exceeded 120°C, and the highest formation rate was observed at around 170°C. When asparagine and glucose were present, the highest levels of acrylamide could be observed, while glutamine and aspartic acid only resulted in trace quantities. The fact that acrylamide is formed mainly
15 from asparagine and glucose may explain the high levels acrylamide in oven-cooked or roasted plant based products such as. Several plant raw materials are known to contain substantial levels of asparagine. In potatoes asparagine is the dominant free amino acid is (940 mg/kg, corresponding with 40% of the total amino-acid content) and in wheat flour asparagine is present at a level of circa 167 mg asparagine/kg flour, corresponding
20 with 14% of the total free amino acids pool (Belitz and Grosch in Food Chemistry - Springer New York, 1999).

Therefore, in the interest of public health, there is an urgent need for food products that have substantially lower levels of acrylamide or, preferably, are devoid of it. In first instance, research activities have been initiated in order to unravel the
25 mechanism of acryl amide formation in food products. So far, the results thereof have not yet led to a satisfactory solution of the problem. Secondly, food companies are investigating the possibilities to avoid the formation of acrylamide by lowering the temperature of the oven cooking and roasting processes. Of course, these adaptations will inherently result in food products with altered taste properties (less Maillard
30 products) and these adaptations raise the risk of an enhanced microbial contamination such as by Salmonella.

The present invention provides a process for the production of a food product

involving at least one heating step, comprising adding one or more enzymes to an intermediate form of said food product in said production process and prior to said heating step in an amount that is effective in reducing the level of amino acids that are present in said intermediate form of said food product and which are involved in the formation of acrylamide during said heating step.

An intermediate form of the food product is defined herein as any form that occurs during the production process prior to the obtention of the final form of the food product. The intermediate form may comprise the individual raw materials used and/or mixture thereof and/or mixtures with additives and/or processing aids, or subsequently processed form thereof. For example, for the food product bread, the intermediate forms comprise for example wheat flour, the initial mixture thereof with other bread ingredients such as for example water, salt, yeast and bread improving compositions, the mixed dough, the kneaded dough, the leavened dough and the partially baked dough.

The food product may be made from at least one raw material that is of plant origin, such as cereal, such as wheat flour or potato. These raw materials are known to contain substantial amounts of amino acids that are involved in the formation of acrylamide during the heating step of the production process. Alternatively, these amino acids may originate from other sources than the raw materials e.g. from protein hydrolysates, such as yeast extracts, soy hydrolysate, casein hydrolysate and the like. A preferred production process is the baking of bread and other baked products from wheat flour and/or flours from other cereal origin. Another preferred production process is the deep-frying of potato chips from potato slices.

Preferred heating steps are those at which at least a part of the intermediate food product, e.g. the surface of the food product, is exposed to temperatures at which the formation of acrylamide is promoted, e.g. 110°C or higher, 120°C or higher temperatures up to. These heating steps may be carried out in ovens (180-220°C), such as for the baking of bread and other bakery products, or in oil such as the frying of potato chips (160-190°C).

The enzymes used in the process of the invention are preferably enzymes that modify the side chains of amino acids that are involved in the formation of acrylamide during the heating step of the production process and whereby the degradation products of said amino acids are not, or at least to a lesser extent, giving rise to the formation of acrylamide in comparison with the undegraded form of the amino acid. Preferably the

enzyme is modifying the side chain of at least one of the amino acids asparagine, glutamine, cysteine, methionine, proline, serine, phenylalanine, tyrosine and/or tryptophane. The enzyme may be added as an enzyme preparation or produced in situ by a microorganism capable of producing said enzyme. Preferably the enzyme preparation is derived from a microorganism and obtained by classical fermentation processes. The microorganism may be a bacterium, a fungus or a yeast. In a preferred embodiment of the invention, the process comprises the addition of asparaginase (EC 3.5.1.1) or glutaminase (EC 3.5.1.2).

In a second aspect, the invention provides food product obtainable by the process of the invention as described hereinbefore. These food products are characterized by significantly reduced acrylamide levels in comparison with the food products obtainable by production processes do that do not comprise adding one or more enzymes in an amount that is effective in reducing the level of amino acids which are involved in the formation of acrylamide during said heating step.

MATERIALS AND METHODS

Acrylamide was measured using the analytical method as indicated in Tareke *et al.*, Chem. Res. Toxicol. 13, 517-522. (2000).

Asparaginase activity was measured according to the method as described by Drinas *et al* Eur. J. Biochem. 151, 591-593. (1985).

The conversion of asparagine was calculated from the amount of ammonia released. Ammonia was estimated by using the ammonia test kit of Sigma (catalogue no. 171 (2002)).

Asparaginase was obtained from *Escherichia coli* (Sigma) and *Erwinia chrysanthemi* (Sigma).

EXAMPLE 1

Preparation of batard type bread.

A dough was prepared from 3000 g of flour (100%), 1740 ml water (58%), 30 g instant yeast (1%), 52.5 g salt (1.75%), 240 mg ascorbic acid (30 ppm) and the indicated amount of asparaginase from *Escherichia coli* (Sigma). The ingredients were

mixed to a dough by a spiral mixer Diosna SP. 12 (2 minutes at speed 1, followed with a mixing time at speed 2 until a total energy input is reached of 72 wh). After this, the complete dough was proofed for 15 minutes at 32°C. Subsequently, dough pieces of 350 g were rounded by hand and proofed for 15 minutes at 32 °C. Hereafter, the dough pieces were rounded and moulded, followed by a final proof of 75 minutes. After proofing, incisions were made in the length of the upper surface of the doughs with a depth of 1 cm. The dough pieces were baked in an oven at 240°C during 30 minutes.

Table 1. Acrylamide and asparagine content of dough and batard breads. The acrylamide of the dough was measured just before baking took place. Every figure is an average of 3 measurements and for each condition, 3 loaves were prepared.

	asparaginase (ppm)**	Acrylamide (ppb)	
		in crumb	in crust
dough	0	< 30* (the inner side)	< 30 (the outer 3 mm)
after baking			
loaf 1	0	45	160
loaf 2	500	< 30	<30

< 30 ppb means not detectable

** based on mg protein

From Table 1 can be concluded that the formation of acrylamide occurs primarily in the crust and not in the crumb. The addition of 500 ppm asparaginase to the dough decreased the level of acrylamide in the bread crust and in the crumb to below the detection level.

CLAIMS

1. Process for the production of a food product involving at least one heating step, comprising adding one or more enzymes to an intermediate form of said food product in said production process and prior to said heating step in an amount that is effective in reducing the level of amino acids that are present in said intermediate form of said food product and which are involved in the formation of acrylamide during said heating step.

2. Process according to claim 1 wherein the food product is made from at least one plant raw material.

3. Process according to claim 2 wherein the plant raw material is cereal flour, preferably wheat flour or potato.

4. Process according to anyone of the preceding claims wherein the enzyme is capable of modifying the side chain of amino acids that are involved in the formation of acrylamide during the heating step of the production process and whereby the degradation products of said amino acids are not, or at least to a lesser extent, giving rise to the formation of acrylamide in comparison with the unmodified form of the amino acid.

5. Process according to claim 4 wherein the enzyme is modifying the side chain of at least one of the amino acids asparagine, glutamine, cysteine, methionine, proline, serine, phenylalanine, tyrosine and/or tryptophane.

6. Process according to any of the preceding claims wherein the enzyme is added as an enzyme preparation or produced in situ by a microorganism capable of producing said enzyme.

7. Process according to claim 7 wherein the enzyme preparation is derived from a microorganism.

8: -- Process according to claim 7 wherein the microorganism is a bacterium, a fungus or a yeast.

5 9. Process according to any of the preceding claims wherein the enzyme is asparaginase (EC 3.5.1.1) or glutaminase (EC 3.4.1.2).

10. A food product obtainable by the process according to anyone of the preceding claims.

ABSTRACT

5 The present invention relates to a process for the production of a food product involving at least one heating step, comprising adding one or more enzymes to an intermediate form of said food product in said production process and prior to said heating step in an amount that is effective in reducing the level of amino acids that are present in said intermediate form of said food product and which are involved in the formation of acrylamide during said heating step. The invention also relates to food products obtained from the process of the invention.